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FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **214887US0XPCT**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/926401

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/01913	**4 March 2000**	**28 April 1999**

TITLE OF INVENTION

MODULAR CELL SUPPORT SYSTEMS FOR THREE-DIMENSIONAL CELL GROWTH

APPLICANT(S) FOR DO/EO/US

Markus OLES, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☒ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Drawings (4 Sheets)
PCT/IB/308
Notice of Priority
Request for Consideration of Documents Cited in the International Search Report

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/926401	PCT/EP00/01913	214887US0XPCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00
	ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x $18.00	$0.00
Independent claims	- 3 =	0	x $84.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
		TOTAL OF ABOVE CALCULATIONS =		$1,020.00
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$0.00
		SUBTOTAL =		$1,020.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
		TOTAL NATIONAL FEE =		$1,020.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				$0.00
		TOTAL FEES ENCLOSED =		$1,020.00
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$1,020.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Telephone: (703)413-3000
Fax: (703)413-2220

Surinder Sachar
Registration No. 34,423

22850

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Oct. 26 2001
DATE

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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

MARKUS OLES ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: 09/926,401 :

FILED: OCTOBER 26, 2001 :

FOR: MODULAR CELL SUPPORT SYSTEMS FOR THREE-DIMENSIONAL CELL GROWTH :

09926401 102601

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to a first examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS

Please cancel Claims 1-9.

Please add the following new claims.

10. (New) A cell support system of porous materials, comprising a plurality of modularly formed segments, said plurality of segments wholly or partly constructed from a plurality of half shells, said plurality of segments further fitted together to give a plurality of larger three-dimensional objects, wherein said cell support system is an artificial capillary network which makes a virtually natural vascularization of a plurality of cells possible.

11. (New) The cell support system as claimed in claim 10, wherein two modularly formed segments form a capillary system by combination of a plurality of half shells.

12. (New) The cell support system as claimed in claim 10, wherein a half shell of a modularly formed segment forms a capillary system by combination with a semipermeable membrane.

13. (New) The cell support system as claimed in claim 10, wherein the plurality of modularly formed segments comprise pores having an average diameter of from 0.5 to 5 μm.

14. (New) The cell support system as claimed in claim 10, wherein an average distance between a plurality of pores in the plurality of modularly formed segments is from 1 to 10 μm.

15. (New) The cell support system as claimed in claim 10, wherein the plurality of modularly formed segments comprise spacers having a height of from 20 to 200 μm.

16. (New) The cell support system as claimed in claim 15, wherein the spacers are hollow and are suitable for liquid transport.

17. (New) A three-dimensional cellular tissue comprising the cell support system as claimed in Claim 10.

18. (New) A method for producing a three-dimensional cellular tissue comprising growing cells in the cell support system claimed in Claim 10.

19. (New) A bioreactor comprising the cell support system as claimed in Claim 10.

REMARKS

Claims 10-19 are active in the present application. Claims 1-9 have been canceled. Claims 10-19 are new claims. Support for the new claims is found in the original claims and in the specification on page 6, line 17 through page 7, line 11. No new matter is believed to have been added. An action on the merits and allowance of claims is solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Stefan U. Koschmieder, Ph.D.
Registration No. 50,238

22850

(703) 413-3000
Fax #: (703)413-2220
DJPER/sjh
I:\atty\SUKOS\214887us-pr.wpd

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Marked-Up Copy
Serial No:

Amendment Filed on:
1-14-2002

IN THE CLAIMS

--Claims 1-9 (Canceled).

Claims 10-19 (New).--

Date Printed: 01/16/2002

41PRTS

09/926401

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CREAVIS Gesellschaft für Technologie und Innovation mbH
PATENTE ♦ MARKEN

Modular cell support systems for three-dimensional cell growth

The present invention relates to artificial cell support systems for three-dimensional cell growth and the use thereof.

The cultivation of animal, human and, increasingly, also plant cells is now employed for a large number of tasks. These include not only scientific purposes and pharmacological investigations but also, increasingly, biotechnological applications such as the production of antibodies and pharmaceuticals. All these applications are based on a two-dimensional growth habit of the cells because only one cell layer (monolayer) can be cultivated with most cell culture techniques.

During serial subcultivation of cells or primary cultures there is often found to be a change in gene expression. This also applies to many immortalized cell lines, which often show only a fraction remaining of their original differentiation. Besides genetic instability, there are other reasons for this differentiation in vitro. The cells in the natural tissue assemblage (in vivo) grow in an environment which is spatially highly structured. This results in different cell interactions and consequently an entirely different cellular activity and proliferation. Another very important feature of the natural tissue assemblage is vascularization. This comprises a dense network of blood vessels (capillaries and venules) which ensure that the cells are supplied with growth factors and oxygen.

This realization has led to refined cell culture techniques which are based more closely on the natural

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environment (in vivo) and include the extracellular matrix (ECM) in the in vitro system.

In vitro cell cultures often grow only two-dimensionally (monolayers). Multilayer growth is desired not only to construct thicker layers but also in order to obtain a cell assemblage capable of functioning, such as, for example, an organ.

Cell assemblages not only have a high cell density but also show interactions between the cells or other tissues. These interactions are epigenetic factors necessary for cellular proliferation and differentiation.

This is why increased efforts have been made recently also to produce multilayer cell cultures (multilayers). The first approaches to this use a three-dimensional growth framework on which the cells can proliferate. The form taken by such frameworks varies very widely. A technique which is now often used is to produce an extracellular matrix from laminin, Matrigel, fibronectin and collagen (e.g.: E.A. Blomme et al., "Influence of extracellular matrix macromolecules on normal human keratinocyte phenotype and parathyroid hormone-related protein secretion and expression in vitro" in Experimental Cell Research, (1998), 238; 1; 204-15). In this technique, the culture vessels are coated with a more or less thin layer of these components. The structure produced in this way is then used as framework for growing various cell types.

Other approaches make use of cellulose foams or hydrogels as frameworks for growing cell cultures, as described in EP 0 451 707-A. The advantage of these foams is the very good surface/volume ratio, i.e. for a small volume a very large surface is provided as adhesion area for cell growth. These growth matrices are often also coated with an extracellular matrix in order to ensure better proliferation and

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differentiation (see, for example: Y. Watanabe et al., "TNF-alpha bifunctionally induces proliferation in primary hepatocytes: role of cell anchorage and spreading" in Journal of Immunology; (1997), pp. 4840-7). Examples of materials employed to produce such foam-like cell supports are cellulose derivatives. The pore formation in these foams is important, because the cells settle in the pores or else nutrients are supplied through small pores in the material. However, only inadequate control of the dimensions of the pores is possible. If the pores are too small, no cells can grow therein, and if the pores are too large unwanted two-dimensional cell growth takes place there. The supply of nutrients which is crucial for growth of the cells, and the transport away of metabolic products likewise depends on a defined pore size distribution. The difficulty of controlling the pore size distribution thus results in the controllability of cell growth being inadequate.

To date it has not been possible to cultivate any functional tissue or organ assemblages using these ideas. These techniques have failed when used for purposes requiring a greater degree of differentiation and thicker cell layers such as, for example, connective tissue or artificial organs. One reason for this is that the supply of thick cell layers with nutrient media and oxygen, as is ensured in vivo by vascularization of the tissue, cannot be guaranteed. Supplying the cells with oxygen and nutrients by intercellular pathways is possible only through a few cells or cell layers.

The use of semipermeable membranes has partly remedied this. A system which makes use of polymer fabrics as support system in conjunction with a perfusion chamber is described, for example, by M. Sittinger et al. in "The International Journal of Artificial Organs" 1997, Vol. 20, No. 1, pp. 57-62. In this case, cartilages are

cultivated in the first step to give a maximally confluent monolayer on large areas of fabric. The cells are then introduced into a perfusion culture system. Cartilage cells are able to grow well in these chambers because an exchange of nutrients and waste products which is adequate for this type of tissue is ensured therein. However, the limits of this technique are reached after only a few layers of cells, so that tissue types which require intensive supplying with nutrients and oxygen cannot be cultivated using this technique.

It is likewise possible to produce an approximately three-dimensional structure by suitably layering individual membranes one on top of the other. The disadvantage of this structure is, however, that it is not self-supporting and can be stacked poorly or only up to a small height, and the nutrient supply through the lengths of membrane on top of one another is difficult to control.

In addition, the individual cell layers are not in mutual contact and thus take the form of two-dimensional layers stacked one on top of the other, and not a three-dimensional structure.

J.C. Hager et al. describe in J. Natl. Cancer Inst., 69, 6 (1982) 1359-66, a system of ordered bundles of hollow fibers for cultivating tumor cells. These fibers serve as surface for cell adhesion and, through pores in the fibers, as supply pathway for providing nutrients and oxygen. It is possible with them to achieve three-dimensional cell growth. Ordered cell growth is not possible owing to the difficulty of controlling the distances between fibers. In addition, the length, diameter and arrangement of the fibers determine the extent and structure of the tissue to be cultivated.

WO 90/02796 and US 5 510 254 describe another possibility for constructing approximately three-

dimensional cell structures. In this case, mesh-like cell support structures, coated where appropriate with growth-promoting substances, are employed. The tissues can be arranged to give superstructures, in which case a cellular connection between the individual layers depends on the distance between them and thus can likewise be influenced only inadequately. Systems of this type are suitable for cell structures with a few layers, but a complex multilayer three-dimensional cell structure cannot be cultivated using these tissues.

Further developments of cell support systems are described in E. Wintermantel, S.-W. Ha, "Biokompatible Werkstoffe und Bauweisen" Springer Verlag 1996, pp. 98-109. There is discussion here in particular of the surface topography and surface functionality of porous supports. However, these support systems likewise do not have defined pore sizes or surface characteristics adapted to the cell type employed and/or the desired purpose of use. Deliberate three-dimensional construction of cellular tissues is not possible using these techniques.

It was thus an object of the present invention to provide a cell support with which three-dimensional cellular tissues can be cultivated in vitro and in vivo.

It has been found that it is also possible to produce complex three-dimensional cellular tissues using a cell support system consisting of modularly formed segments of a porous material.

The present invention therefore relates to a cell support system of porous material, where the cell support system consists of modularly formed segments which are wholly or partly constructed from half shells.

The porosity of the modularly formed segments can be adapted specifically to suit the cell type used. The modularly formed segments may have, depending on the cell type, pores with an average diameter of from 0.5 to 5 µm. The distribution of the pores is advantageously chosen so that between one and three pores are available per grown cell for supplying the cells, i.e. the average distance between the pores in the segments is advantageously from 1 to 10 µm. The segments of the cell supports have wholly or partly a porous structure, the target being cell growth preferably only at the porous points in the segments.

The nonporous points in the segments can, owing to the reduced cell growth there, be employed for attachment purposes or the like.

The cellular tissue cultivated on the cell support systems according to the invention is, because of the excellent vascularization, capable of proliferation in vitro and in vivo. The modular form of the segments makes it possible to construct cell support systems of virtually any shape and complexity. The optional connection between two or more segments makes it possible to cultivate coherent cell and tissue cultures of virtually any size.

Cell support systems according to the present invention make it possible to construct three-dimensional cellular tissues in which all the cells can be supplied with nutrient solution and oxygen through a porous and thus microstructured surface.

The cells on the cell support systems according to the invention are supplied through a capillary system which can be formed by combining the half shells of in each case two modularly formed systems. The segments can be combined in such a way that a closed hollow article, i.e. a capillary system, is produced from the two half

shells. Combination of two segments can be simplified by appropriate holding pins. The capillaries preferably have a diameter of 20-70 µm.

A system of this type makes it possible to distribute released growth factors in the entire cell culture and thus make differentiation of the tissue possible. It is possible with the present invention to ensure a continuous flow out and in of nutrients, metabolic products, oxygen and growth factors to the cellular tissues.

Cell growth and cell differentiation are considerably influenced by the surface topography of the cell support. The exchange of nutrients and the distribution of the cells on the surface is determined by the nature and topography of the microstructure, i.e. in the present case by the porosity of the surface. Most applications are in this case limited by diffusion of the metabolic activity of the tissue. With the present invention, owing to the good nutrient supply, as the metabolic activity increases there is also an increase in the vascularization of the tissue, and thus a reduction in the necessary diffusion pathways.

It is an essential feature of the present invention that the cell support systems consist of formed segments which make a modular construction of an integrated system possible.

Examples of suitable materials for the cell support systems according to the invention are polycarbonate, poly(methyl methacrylate), polyurethane, polyamide, PVC, polyethylene, polypropylene, polystyrene or polysulfonate, and blends or copolymers thereof.

Fixation of two segments to form a capillary system can take place by adhesive or microwave or high-frequency techniques. It is self-evident that this must take

place in such a way that the pores of the material are impaired only slightly, if at all.

The cell support systems, whether individual segments or preformed capillary systems, can also be connected together. This can be achieved by using spacers which are advantageously fixed to the segments during production thereof. The spacers additionally set a constant distance between individual segment layers, so that cells are able to grow here too. The modularly formed segments preferably have spacers with a height of from 20 to 200 µm. If the spacers are hollow and suitable for liquid transport, it is possible in this way to guide the nutrient solution through the entire system.

The modular design of the segments mimics the natural environment of the cells, so that proliferation, differentiation or performance of the physiological functions of the cells takes place for as long as the cells can be supplied with nutrient solution through the porous material. This supply usually takes place through from 2 to 20 cell layers, with the number of cell layers supplied depending greatly on the metabolism of the cells. Liver and kidney cells must be cultivated on cell support systems with small spacings (20-40 µm) because they require a large blood supply even in the body. On the other hand, the distance between the cell support systems can be very large, up to 200 µm, for fibroblasts and cartilage cells.

The individual segments can be produced by microsystem techniques. An example of a suitable process is the LIGA process which is a structure-forming process based on X-ray lithography, electroplating and molding. It is then possible, using the mold inserts produced by the LIGA technique, to produce as many copies as desired by injection molding, reaction injection molding or embossing processes from various plastics with great

trueness to detail and at relatively low cost. The pores can be introduced into the material by suitable projections on the mold inserts.

Fig. 2 shows by way of example the structure of a cell support according to the invention consisting of two segments. One segment consists of a central supply tube with perpendicular branches at periodically repeating intervals. These branches form a capillary system. The surfaces of the segments are provided with small pores which have a diameter of 0.5-5 µm, depending on the cell type used. The average distance between the pores is from 1 to 10 µm, and the distance between the branches (L1) may be between 20 and 200 µm, appropriate for the cell type.

The nutrient medium is delivered actively or passively, by an appropriate gradient, through the central supply tube. The distribution of the nutrient medium and of the respiratory gases to the tissue is ensured by diffusion. The nutrient circulation is designed so that the medium is able to run out again through an outflow and be returned to the circulation or collected for reprocessing/disposal.

The individual segments have a modular structure so that they can be assembled with accurate fit to produce larger three-dimensional objects. This results in an artificial capillary network which makes virtually natural vascularization of the cells possible. The segments suitably have appropriate spacers as plug-in devices in order to allow two segments to be connected simply and with accurate fit.

In order to adjust the required distances between the segments according to the invention, they are provided with spacers. The spacers expediently act as plug-in device for fixing two segments (AH in Fig. 3). Flow in and out is likewise designed to allow a liquid-carrying

connection between the individual segments. Spacers designed to be hollow can be employed to connect the flows in and out of segments.

The segments can also be stacked offset relative to one another.

After the cell support has been constructed from the individual segments, the required cell types can be applied to the latter. The system is for this purpose placed in a roller bottle with a cell suspension of high density. The system remains in this bottle with a moderate speed of rotation of the roller bottle until sufficient cells have become fixed to the surface. This is typically complete after 3 to 8 hours. The system is then transferred, preferably under sterile conditions, into a Petri dish, and fresh medium is continuously pumped through the supply connections of the segments and through the cell supports. After a few days, a multilayer cellular tissue forms on the segment surfaces and thus between the channel walls.

Alternatively, the cellular tissue can also be constructed stepwise. Firstly one plane of the cell supports according to the invention is incubated with cells. After a cell layer has grown on this lowest plane, the system is extended stepwise by one cell support layer in order to allow a cell layer to grow onto this too. The successive procedure has the advantage that a cell type can be forced to differentiate diversely through different distances between the segments or the support layers. Diverse differentiation of a cell type is important, for example, for skin cells. Distances between segments of 3-6 cell layers have proven suitable in practice.

The cell supports according to the invention allow the cells to be supplied with nutrients satisfactorily. This can be achieved by branching of the segments.

Fig. 4 a to e shows an example of a design of a system of this type, based on a honeycomb structure. Nutrient medium is pumped into this system through an inlet. The medium is able to run out through the outflow and be returned to the circulation or collected for reprocessing/disposal. The surface of the segments is provided with small pores of a size and distribution as described above. An artificial capillary network is also produced in this variant of the design by combination of the segments.

The diameter of the individual honeycomb elements (width of the opening) depends on the cell type used and may be between 70 and 180 µm. In order to ensure optimal supply to the cells, the next honeycomb cell support can be stacked rotated by 90 degrees (Fig. 4 c) on top of the preceding cell support.

As described for the ladder-like structure, it is also possible with honeycomb segments to construct a three-dimensional cell culture. In this case too, appropriately designed plug-in connections between the honeycomb elements allow layer-overlapping cell growth (Fig. 4 e).

The honeycomb cell supports are, as outlined in Fig. 1, constructed from two half shells which are firmly connected together or from one half shell and one membrane.

The cell supports according to the invention can also be constructed from fairly shallow segments. Fig. 5 shows diagrammatically the construction of a cell support of this type in a pyramidal design in plan (Fig. 5 a) and side view (Fig. 5 b and c). The segments are arranged periodically in parallel rows (Fig. 5 c and d). A distance is left between the rows, preferably of half the base area of a pyramid. The individual rows of segments may in turn be connected together by, where

appropriate spacers suitable for liquid transport. Nutrient medium is pumped through the elements via an inlet. The medium can run out through an outflow and be returned to the circulation or collected for reprocessing/disposal. The surfaces of the pyramids are provided with small pores of the size and distribution as described. The pyramids themselves are hollow and open on the base area and thus likewise form a half shell. The side view in Fig. 5 c shows the connection of two segments to form a closed cell support system.

A cell culture with pyramidal cell support segments according to the invention can be constructed as follows: some pyramidal segments are placed as base element on the bottom of a suitable cell culture system. Further segments can then be positioned above these structures. The cell supports are produced by the combination of segments (see side view in Fig. 5 c). The segments can be fitted together so that there is a space in which the cells can grow between the surfaces of the pyramids.

The advantage of this structure is that the geometric dimensions of the elements are independent of the cell type chosen. Only the distance between the layers and the pore diameter of the elements need to be adapted to the cell type used. In order to maximize the cell density and achieve a small dead volume within the pyramids, i.e. the supply elements, it is advisable for the height of the individual pyramidal elements to be small by comparison with their base area. The cell supports shown in Fig. 5 d have the following dimensions:

Height of pyramid a1:	20 - 40 µm
Height of base area a2:	20 - 40 µm
Width of segments a3:	150 - 300 µm
Length of segments a5:	integral multiple of a3
Distance between cell supports a4:	50 - 300 µm

As an alternative to the cell support systems constructed from two half shells, these can also be formed by combining a half shell of a modularly formed segment with a semipermeable membrane to construct a capillary system. In this case, a permeable membrane is clamped onto the rear side of a segment. The projecting parts of the membrane can be removed by suitable etching processes. This technique has the advantage that it is unnecessary to assemble two segments with accurate fit. Semipermeable membranes such as Goretex, Simpatex or ceramic membranes are suitable for this purpose. Plasma etching has proved to be the preferred etching process. This is a dry etching variant used to produce structures in the µm range. After the membrane has been applied to the rear side of a segment by a phase inversion process, the projecting parts of the membrane are etched away in a plasma reactor with plasma gases such as F_2, Cl_2, CF_3^-/F, CCl_3^+/Cl and O_2. This embodiment of the present invention also eventually produces closed cavities or capillaries. The pore size and distribution of the membranes correspond to those of the segments with an average distance of from 1 to 10 µm and an average diameter of from 0.5 to 5 µm.

The present invention further relates to the use of the three-dimensional cell support systems for bioreactors and for cultivating eukaryotic or organic stem cells.

Important stem cells are hepatocytes, kidney cells, endothelial cells, epithelial cells or myocytes.

The cell cultures used in biotechnology to produce hormones, cytokines and other pharmaceuticals which can be produced by genetic manipulation have had their genetic material modified so that they are able to produce the required substances. Since these cells have to date been cultivated almost exclusively in two-

dimensional cultures, these cells differentiate very rapidly. The consequence of this is that the required substances are not produced by the cell for very long, and the cells have to be replaced or the genetic material of the cells must be modified again. The use of the three-dimensional cell supports according to the invention for the cultivation has the advantage that the phenotype of the cells employed is substantially retained and differentiation begins later or not at all. It is possible in this way to achieve crucial production advantages.

It is thus also possible to synthesize human proteins by using cell supports according to the invention optimized for human cell types. This means that the structure and, in particular, the folding of the synthetic proteins correspond to the natural proteins in the human body.

Since the cells adhere to the cell supports according to the invention and are not present in a suspension, the proteins or other substances produced by the cells can be continuously removed through the nutrient supply circulation. With nonadherent systems, this is possible only by filtration or centrifugation of the suspensions. This makes it possible, for example to construct cell cultures as implant or even artificial hybrid organs.

The artificial production of replacement organs still encounters very great difficulties. Clinical approaches to a solution to date have been only for an artificial liver (H.G. Koebe; F.W. Schildberg in "Die künstliche Leber - ein Zwischenbericht.", Wiener klinische Wochenschrift, 110; 16; 551-563; 1998). In this case, a suspension of hepatocytes is kept in a perfusion chamber which is connected to the patient's blood circulation and is able to take over the function of the defective liver. This technique can to date be used

only for acute liver failure because the limited survival time and the altered phenotype of the cultures precludes prolonged use thereof at present.

The use of cell support systems according to the invention has the advantage that the hepatocytes are not in suspension but are able to grow in an organotypical manner. This ensures that the hepatocytes achieve a degree of differentiation like that present in vivo.

Adequate supply of the hepatocytes is possible by use of the cell support systems according to the invention and the vascularization possible in this way. The individual segments are connected in such a way that there is only one inlet and one outlet. To improve handling and to protect from infections, the system is closed by an external encapsulation. A patient's blood circulation can then be connected via the inlet and outlet which pass to the outside. The cells in the reactor then take over the function of the liver. It is also possible with this technique to construct other artificial organs such as, for example, a kidney.

Human kidney cells can even now be maintained well in culture. Functional use of these cells for dialysis has, however, to date been frustrated by the reproduction of nephrons in conjunction with functionally differentiated kidney cells. It is possible, by combining microsystem techniques and cell culture techniques, to reproduce such functional kidney units. However, two separate circulatory systems are necessary for this, one system for the urine and one system for the blood circulation. Suitable encapsulation must also be provided in this case.

Further areas of use of the cell supports according to the invention are Langerhan's cells of the pancreas, whose function is restricted in diabetics. Insulin can

be produced artificially by putting healthy cells of this type on a framework of cell supports. The cell supports are connected to the patient's blood circulation. The system must be closed by an external encapsulation as on use as organ replacement.

The reproduction of artificial tissue and tissue replacement on cell supports according to the invention has crucial advantages in toxicity testing. Encapsulation is unnecessary for reproduction of the skin. To simulate the anatomical pattern it is necessary when cultivating artificial skin for the blood supply to decrease steadily toward the dermis. Technically, this can be achieved by increasing distances between the segments in the cell culture. Since the artificial vascularization is, owing to this manner of construction, located in accurately defined cell layers, this can also be used for penetration tests. However, for such studies, the supply of the elements in the cell culture must be stratified so that nutrient medium can be taken for analysis only in the required cell layer.

The use of cell supports according to the invention has advantages in particular in the production of models of disease. For this purpose, the cells which have the characteristic features of the disease at the cellular level are placed in a cell culture and maintained in a 3D culture by segments. This technique results in the cells remaining in the "pathological" physiological state for longer and not redifferentiating so quickly. Such models are used mainly in the drugs industry, which is able to test new pharmaceuticals on such models. In addition, such models may make a crucial contribution to the understanding of some diseases.

Patent claims:

1. A cell support system of porous materials, which consists of modularly formed segments which are wholly or partly constructed from half shells.

2. A cell support system as claimed in claim 1, wherein in each case two modularly formed segments form a capillary system by combination of the half shells.

3. A cell support system as claimed in claim 1, wherein a half shell of a modularly formed segment forms a capillary system by combination with a semipermeable membrane.

4. A cell support system as claimed in any of claims 1 to 3, wherein the modularly formed segments have pores with an average diameter of from 0.5 to 5 μm.

5. A cell support system as claimed in any of claims 1 to 4, wherein the average distance between the pores in the modularly formed segments is from 1 to 10 μm.

6. A cell support system as claimed in any of claims 1 to 5, wherein the modularly formed segments have spacers with a height of from 20 to 200 μm.

7. A cell support system as claimed in claim 6, wherein the spacers are hollow and are suitable for liquid transport.

8. The use of the cell support systems as claimed in any of claims 1 to 7 for cultivating eukaryotic or organic stem cells.

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9. The use of the cell support systems as claimed in any of claims 1 to 7 for bioreactors.

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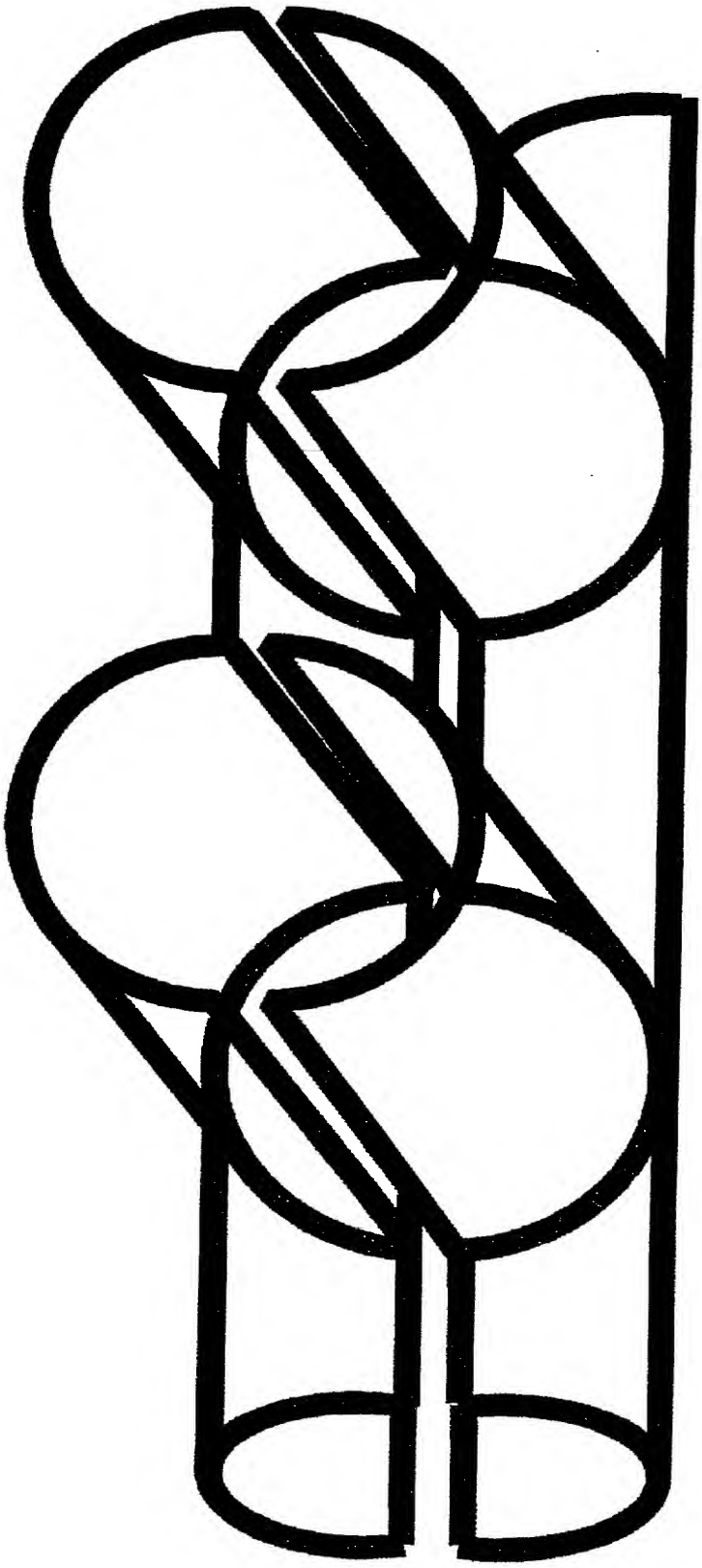

Fig. 1

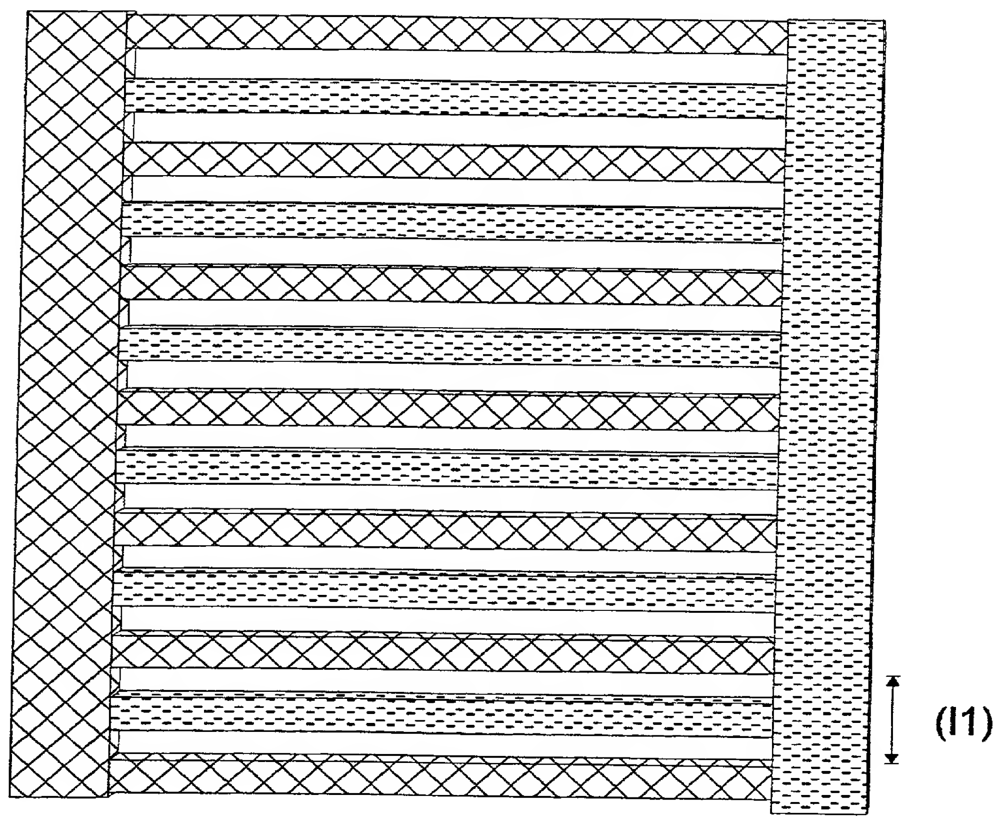

Fig. 2

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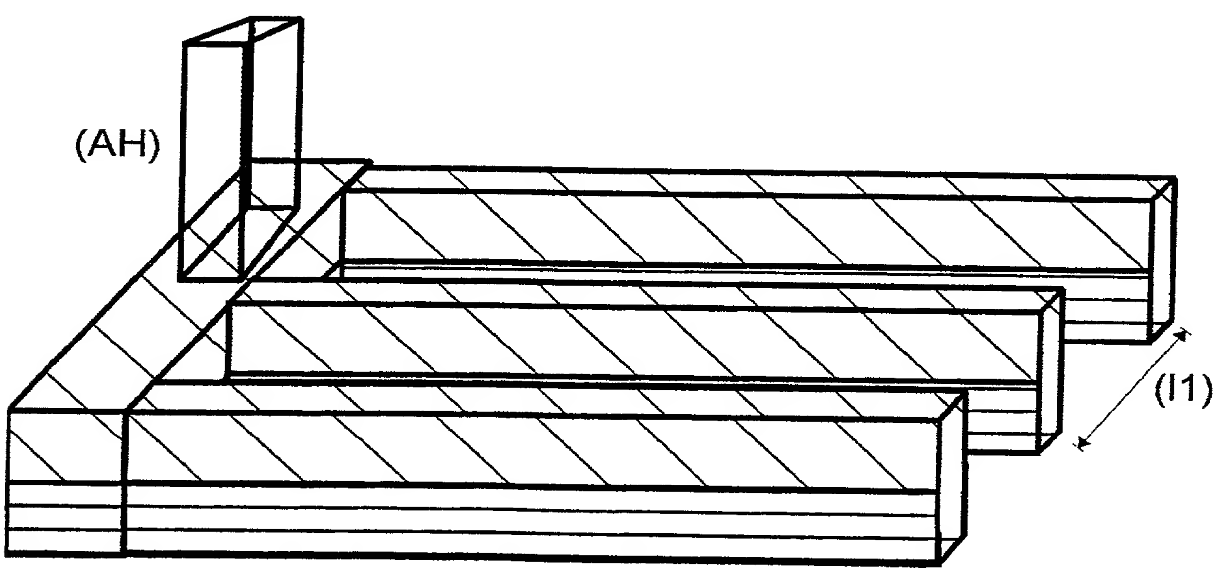

Fig. 3

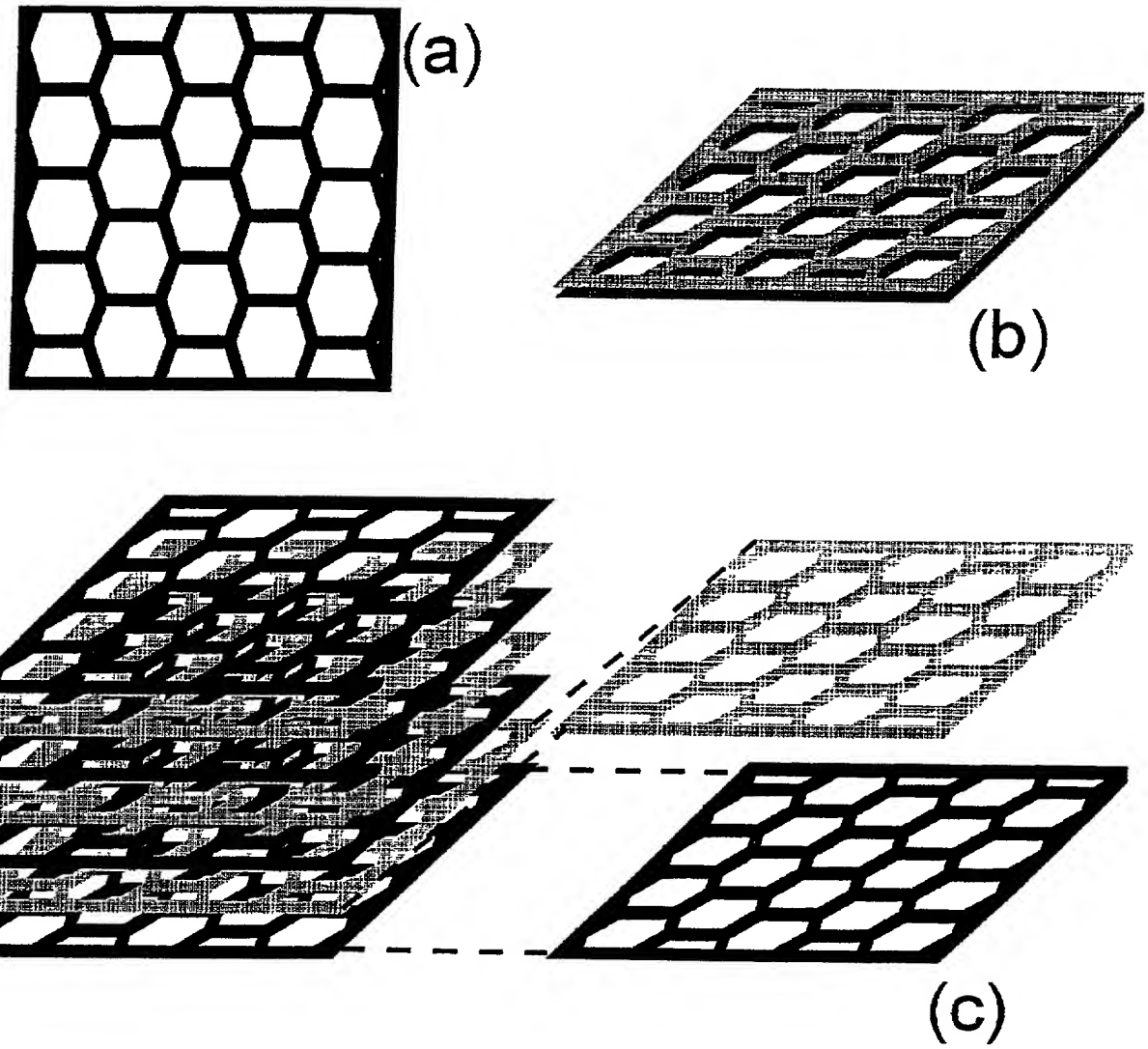

Fig. 4

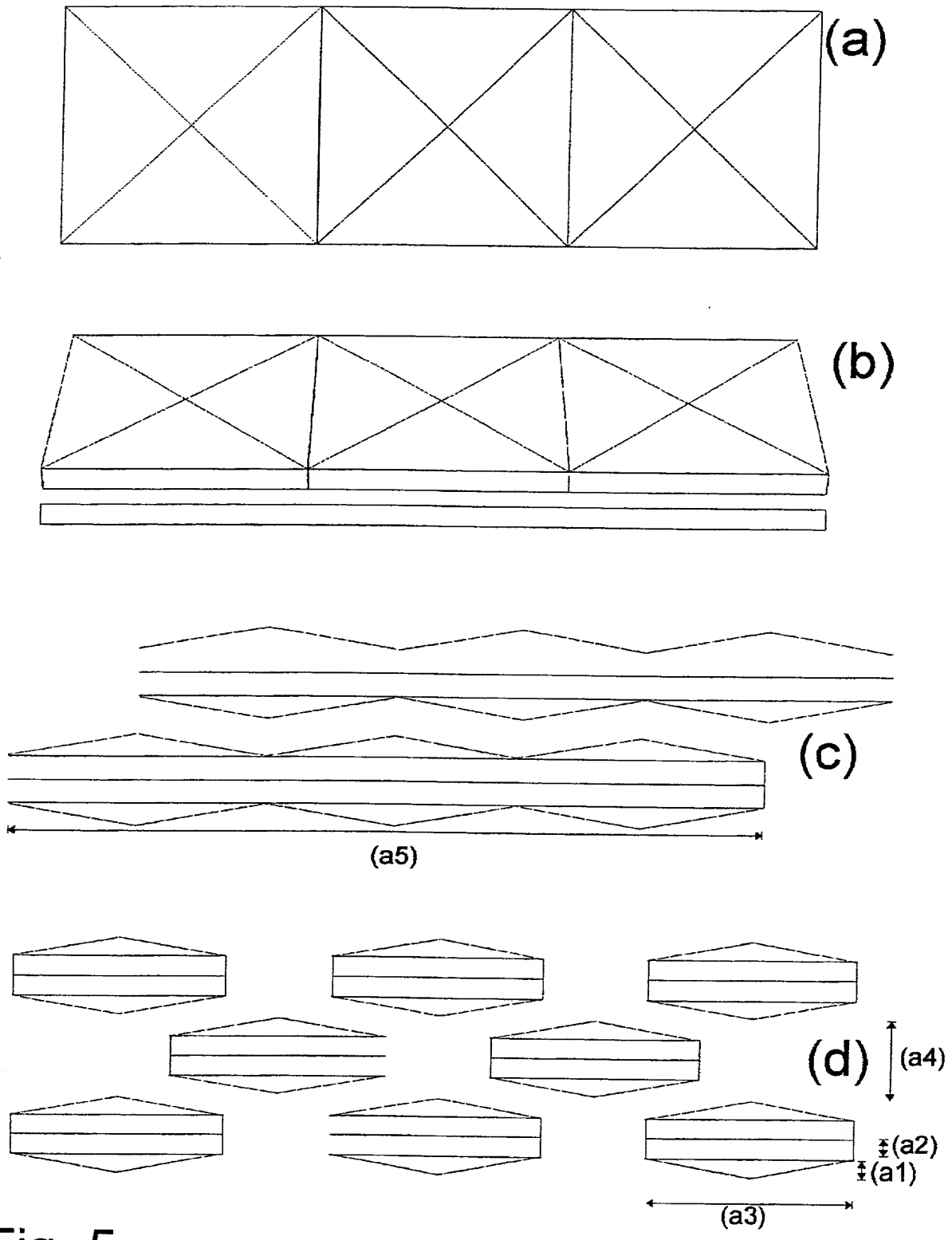

Fig. 5

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Declaration and Power of Attorney for Patent Application
Erklärung für Patentanmeldungen mit Vollmacht

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German Language Declaration

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_____ abgeändert (falls zutreffend).

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Modular cell support systems for three-dimensional cell growth

the specification of which:

☐ is attached hereto.

☒ was filed on March 4, 2000

as United States Application Number or PCT International Application Number

PCT/EP00/01913

_____ and was amended on

_____ (if applicable)

Ich bestätige hiermit, daß ich den Inhalt der oben angegebenen Patentanmeldung, einschließlich der Ansprüche, die eventuell durch einen oben erwähnten Zusatzantrag abgeändert wurde, durchgesehen und verstanden habe.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Ich erkenne meine Pflicht zur Offenbarung jeglicher Informationen an, die zur Prüfung der Patentfähigkeit in Einklang mit Titel 37, Code of Federal Regulations, § 1.56 von Belang sind.

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Prior foreign application(s)
(Frühere ausländische Anmeldungen)

(Number) (Nummer)	(Country) (Land)	(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	Priority claimed / Priorität beansprucht: Yes / Ja	No / Nein
199 19 242.1	Germany	April 28, 1999	☒	☐
			☐	☐

☐ Ich Beanspruche hiermit Prioritätsvorteile unter Title 35, US-Code, § 119(e) aller US-Hilfsanmeldungen wie unten aufgezählt.

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Status) (patented, pending, abandoned) (Status) (patentiert, schwebend, aufgegeben)
PCT/EP00/01913	March 4, 2000	pending

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

Norman F. Oblon, Reg. No. 24,618; Marvin J. Spivak, Reg. No. 24,913; C. Irvin McClelland, Reg. No. 21,124; Gregory J. Maier, Reg. No. 25,599; Arthur I. Neustadt, Reg. No. 24,854; Richard D. Kelly, Reg. No. 27,757; James D. Hamilton, Reg. No. 28,421; Eckhard H. Kuesters, Reg. No. 28,870; Robert T. Pous, Reg. No. 29,099; Charles L. Gholz, Reg. No. 26,395; William E. Beaumont, Reg. No. 30,996; Jean-Paul Lavalleye, Reg. No. 31,451; Stephen G. Baxter, Reg. No. 32,884; Richard L. Treanor, Reg. No. 36,379; Steven P. Weihrouch, Reg. No. 32,829; John T. Goolkasian, Reg. No. 26,142; Richard L. Chinn, Reg. No. 34,305; Steven E. Lipman, Reg. No. 30,011; Carl E. Schlier, Reg. No. 34,426; James J. Kulbaski, Reg. No. 34,648; Richard A. Neifeld, Reg. No. 35,299; J. Derek Mason, Reg. No. 35,270; Surinder Sachar, Reg. No. 34,423; Christina M. Gadiano, Reg. No. 37,628; Jeffrey B. McIntyre, Reg. No. 36,867; William T. Enos, Reg. No. 33,128; Michael E. McCabe, Jr., Reg. No. 37,182; Bradley D. Lytle, Reg. No. 40,073; and Michael R. Casey, Reg. No. 40,294, with full powers of substitution and revocation.

Postanschrift:

Send Correspondence to:
OBLON, SPIVAK, MCCLELLAND, MAIER & NEUSTADT, P.C.
FOURTH FLOOR
1755 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202 U S A

Telefonische Auskünfte:
(Name und Telefonnummer)

Direct Telephone calls to: *(name and telephone number)*

(703) 413-3000

Vor- und Zuname des einzigen oder ersten Erfinders	Full name of sole or first inventor Dr. Markus OLES
Unterschrift des Erfinders Datum	Inventor's signature Date Markus Oles 09. NOV. 2001
Wohnsitz	Residence Hattingen, Germany DE
Staatsangehörigkeit	Citizenship German
Postanschrift	Post Office Address Im Mühlenwinkel 2, D-45525 Hattingen,
	Germany
Vor- und Zuname des zweiten Miterfinders (falls zutreffend)	Full name of second joint inventor, if any Dr. Dierk LANDWEHR
Unterschrift des zweiten Erfinders Datum	Second inventor's signature Date Dierk Landwehr 24/10/01
Wohnsitz	Residence Dülmen, Germany DE
Staatsangehörigkeit	Citizenship German
Postanschrift	Post Office Address Haverlandweg 150, D-48249 Dülmen,
	Germany

(Im Falle dritter und weiterer Miterfinder sind die entsprechenden Informationen und Unterschriften hinzuzufügen)

(Supply similar information and signature for third and subsequent joint inventors)

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Vor- und Zuname des dritten Miterfinders (falls Zutreffend) 300	Full name of third joint inventor, if any Dr. Beate KOSSMANN 09. NOV. 2001
Unterschrift des dritten Erfinders Datum	Third inventor's signature Date Beate Kossmann
Wohnsitz	Residence Hagen, Germany DE
Staatsangehörigkeit	Citizenship German
Postanschrift	Post Office Address Ribbertstraße 13, D-58091 Hagen,
	Germany
Vor- und Zuname des vierten Miterfinders (falls Zutreffend)	Full name of fourth joint inventor, if any
Unterschrift des vierten Erfinders Datum	Fourth inventor's signature Date
Wohnsitz	Residence
Staatsangehörigkeit	Citizenship
Postanschrift	Post Office Address
Vor- und Zuname des fünften Miterfinders (falls Zutreffend)	Full name of fifth joint inventor, if any
Unterschrift des fünften Erfinders Datum	Fifth inventor's signature Date
Wohnsitz	Residence
Staatsangehörigkeit	Citizenship
Postanschrift	Post Office Address
Vor- und Zuname des sechsten Miterfinders (falls Zutreffend)	Full name of sixth joint inventor, if any
Unterschrift des sechsten Erfinders Datum	Sixth inventor's signature Date
Wohnsitz	Residence
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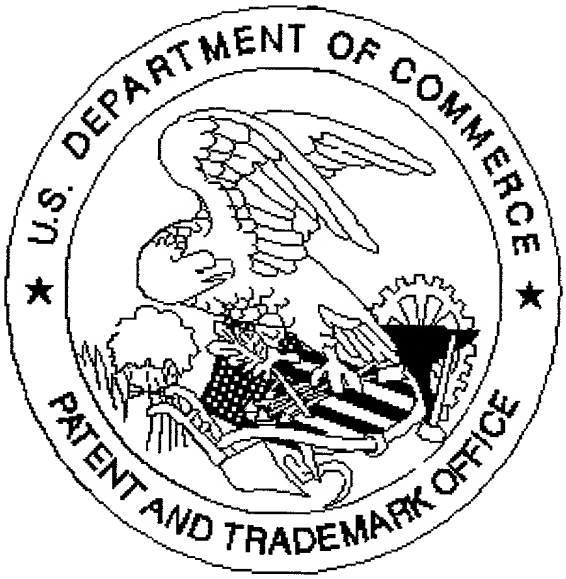

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